if a virulence-associated member, sequencing the entire pathogenicity island, and identifying genes within this sequence.

78. A method of determining whether a particular bacterial strain harbours a type III secretion system involved in pathogenicity, comprising:

designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;

amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers to determine the presence of any *lcrD*-like genes in said bacterial strain;

if amplified successfully, sequencing the *lcrD*-like gene; and

determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes.

REMARKS

The above-identified application is being entered into the National Phase from PCT application no. PCT/EP99/10297.

Specification and Sequence Listing

An inadvertant error on page 38 (Table 3) which recites position 31773 as the end of the open reading frame for *orf14* has been corrected to show position 31818. As set forth below, correcting this error is obvious.

Applicants respectfully request amendment of SEQ ID NO:69 and SEQ ID NO:70 as set forth on substitute sheets 111, 113, and 114 submitted herewith pursuant to 37 CFR §§ 1.825.

In the original submission of the Sequence Listings, the Sequence Listing program completely ignored the stop codon at position 396. Although the sequence shown in Fig. 5 is correct, Table 3 states that *orf14* is encoded in the complementary strand from position 31773-33005. Although position 33005 indicates the correct start codon for this open reading frame, the end of the open reading frame is incorrectly stated. It should actually be at position 31818 – where the first stop codon (at position 396) is encountered.

Correcting this error is obvious. Given a properly stated start codon, and a correct DNA sequence, anyone would realize that the open reading frame MUST end where the first, in-frame,

stop codon in the sequence is encountered. This is obviously at position 396, and was clearly the intention of the Applicants at the time of filing the international application.

Three replacement sheets are provided as required under 37 CFR §§ 1.825. The error on page 111 has been rectified to indicate the number of nucleotides in SEQ ID NO:69 as being 1188. The error on page 113 has been rectified to indicate that the open reading frame does not extend past the stop codon at position 396. In addition, the number of amino acids in SEQ ID NO:70 has been rectified as being 395. The error on page 114 has been rectified to indicate the last amino acid in the open reading frame as being His395.

The corrected sequence listing for SEQ ID NO:69 and 70 does not go beyond the disclosure apparent to anyone from Fig. 5 and Table 3 of the International Application as filed.

The complete sequence listing is provided on a computer diskette. It is identical to the original written sequence listing in conjunction with the aforementioned corrections to SEQ ID NO: 69 and 70.

Claims

Claims 1-29 were cancelled. New claims 30-78 were added for the following reason: to put the claims in conformity with U.S. practice.

No new matter has been introduced.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with Markings to Show Changes Made"**. Applicants respectfully request that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES

IN THE SPECIFICATION:

Table 3 appearing on page 38 has been amended as follows:

Table 3

Names	Coding sequence from/to (with reference to Fig. 5)	Coding DNA strand	SEQ ID NO:	Homologous genes (from *Yersinia*, unless otherwise specified)
Class II ORFs which putatively code for effector proteins				
bopN	11906/13003	complement	41	*YopN* (= *lcrE*)
orf1	6160/6747	direct	43	None
orf2	10752/11120	complement	45	None
orf3	11117/11527	complement	47	None
orf4	11532/11909	complement	49	None
orf5	13002/13784	direct	51	None
orf6	13806/14081	direct	53	None
orf7	14630/15571	direct	55	None
orf8	15601/16803	direct	57	None
orf9	16827/17288	direct	59	*BcrH*
orf10	17293/17814	direct	61	*pcr4* (*Pseudomonas aeruginosa*)
orf11	29412/29591	complement	63	None
orf12	29555/30529	complement	65	None
orf13	30631/31776	direct	67	None
orf14	[31773]31818/33005	complement	69	None
orf15	32370/33014	direct	71	None

IN THE SEQUENCE LISTING:

IN THE CLAIMS:

Claims 1-29 have been cancelled. New claims 30-78 have been added as follows:

30. An isolated polypeptide comprising an amino acid sequence which has at least 75% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72 over its entire length.

31. The polypeptide as claimed in claim 30 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72.

32. An isolated polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.

33. An isolated polypeptide comprising a fragment of at least 7 consecutive amino acids of the polypeptide as claimed in any one of claims 30 to 32, wherein the fragment comprises an epitope.

34. The polypeptide of claim 33, wherein the fragment is immunogenic.

35. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 75% identity to the amino acid sequence of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72 over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.

36. An isolated polynucleotide comprising a nucleotide sequence that has at least 75% identity to a nucleotide sequence, encoding a polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72, over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.

37. An isolated polynucleotide which comprises a nucleotide sequence which has at least 75% identity to that of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.

38. The isolated polynucleotide as claimed in claim 35 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length.

39. The isolated polynucleotide as claimed in claim 36 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length.

40. The isolated polynucleotide as claimed in claim 37 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length.

41. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.

42. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71.

43. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72, obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 or a fragment thereof.

44. An expression vector comprising an isolated polynucleotide according to any one of claims 35-43.

45. A recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 35-43.

46. A host cell comprising the expression vector of claim 44 or a subcellular fraction or a membrane of said host cell.

47. A process for producing the polypeptide of claim 30 comprising the steps of culturing a host cell of claim 46 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

48. A process for expressing a polynucleotide of any one of claims 35-43 comprising transforming a host cell with an expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

49. A vaccine composition comprising an effective amount of the polypeptide of claim 30 and a pharmaceutically acceptable carrier.

50. A vaccine composition comprising an effective amount of the polypeptide of claim 31 and a pharmaceutically acceptable carrier.

51. A vaccine composition comprising an effective amount of the polypeptide of claim 32 and a pharmaceutically acceptable carrier.

52. A vaccine composition comprising an effective amount of the polypeptide of claim 33 and a pharmaceutically acceptable carrier.

53. A vaccine composition comprising an effective amount of the polypeptide of claim 34 and a pharmaceutically acceptable carrier.

54. The vaccine composition of claim 49, wherein the polypeptide has an amino acid sequence selected from the group consisting of: SEQ ID NO:42, 46, 48, 50, 52, 54, 56, 58, 60 and 62.

55. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 35 to 43 and a pharmaceutically acceptable carrier.

56. The vaccine composition according to any one of claims 49-55, wherein said composition comprises at least one other *Bordetella pertussis* antigen.

57. An antibody immunospecific for the amino acid sequence of claim 30 or 31.

58. An antibody immunospecific for the polypeptide of claim 32.

59. An antibody immunospecific for the fragment of claim 33.

60. An antibody immunospecific for the fragment of claim 34.

61. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 30, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

62. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 31, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

63. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 32, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

64. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 33, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

65. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 34, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

66. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 30 and a suitable pharmaceutical carrier.

67. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 31 and a suitable pharmaceutical carrier.

68. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 32 and a suitable pharmaceutical carrier.

69. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 33 and a suitable pharmaceutical carrier.

70. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 34 and a suitable pharmaceutical carrier.

71. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polynucleotide of claims 35-43.

72. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 30.

73. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 31.

74. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 32.

75. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 33.

76. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 34.

77. A method of identifying virulence genes from a pathogenicity island containing a type III secretion system from pathogenic strains of bacteria, comprising:

designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;

amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers of *lcrD*-like genes present in said pathogenic strain of bacteria;

sequencing the *lcrD*-like gene;

determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes; and

if a virulence-associated member, sequencing the entire pathogenicity island, and identifying genes within this sequence.

78. A method of determining whether a particular bacterial strain harbours a type III secretion system involved in pathogenicity, comprising:

designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;

amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers to determine the presence of any *lcrD*-like genes in said bacterial strain;

if amplified successfully, sequencing the *lcrD*-like gene; and

determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes.